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(71) Applicant: MICHIGAN STATE UNIVERSITY [US/US]; East Lansing, MI (US).

(72) Inventors: NAIR, Muraleedharan; 3934 East Sunwind Drive, Okemos, MI 48864 (US). MOMIN, Rafikali; 24 Porter Drive, Guelph, Ontario N1L 1M4 (CA).

(74) Agent: CARTER, Charles, G.; Foley & Lardner, 777 East Wisconsin Avenue, 33rd Floor, Milwaukee, WI 53202 (US).

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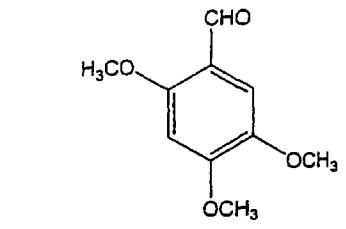
(54) Title: METHOD OF TREATING INFLAMMATION AND INFLAMMATION-RELATED PAIN

(57) Abstract: A method for treating cycloxygenase enzyme inflammation and inflammation pain is disclosed. In one embodiment, this method comprises the step of treating an inflammation patient with a specific amount of carrot seed or carrot seed extract, wherein inflammation is reduced and pain is decreased.



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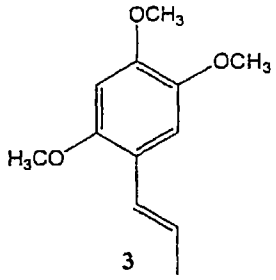

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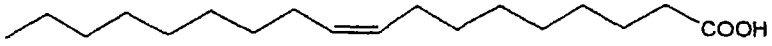

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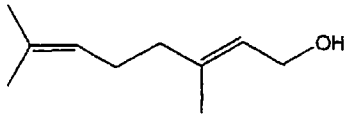

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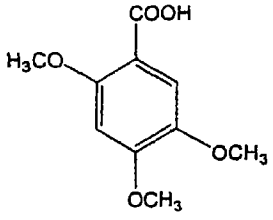

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1 = 2,4,5-trimethoxybenzaldehyde
2 = Oleic acid
3 = trans-asarone
4 = Geraniol
5 = 2,4,5-trimethoxybenzoiacid

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METHOD OF TREATING INFLAMMATION AND INFLAMMATION-RELATED PAIN

CROSS-REFERENCE TO RELATED APPLICATION

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STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

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BACKGROUND OF THE INVENTION

Carrot (*D. carota* L.) is an annual or biennial herb cultivated throughout the temperate regions of the world. Carrots are one of the important food crops in the world, and are used extensively for canned, frozen and dehydrated products. Even though carrot is widely used as a vegetable, other parts of this plant are used in traditional medicine for the treatment of a broad spectrum of ailments. Carrot seeds, commonly known as carrot fruits, are well known for its use as carminative, diuretic, stimulant, and in the treatment of digestive disorder (Volak, et al., 1984). The essential oil from the seeds was also studied for its hypotensive, cardiac, anticonvulsant and anti-fertility activities (Kamboj, 1988; Dhar, 1990; Chopra, et al., 1958; Halim, et al., 1988). Seed oils of some selected varieties were reported as an antibacterial (Syed, et al., 1986) and fungicidal (Guerin and Reveillere, 1985; Dwivedi, et al., 1991) agents. Carrot seed oil is widely used as flavoring agent in food products, grape wine, nonalcoholic beverages (Bodrug, 1982) and in perfumery (Guenther, 1950).

The conversion of arachidonic acid to prostaglandins, catalyzed by cyclooxygenase enzymes COX-I and COX-II, is well documented (O'Banion, 1999). The COX-I enzyme is constitutively expressed in many tissues. COX-II enzyme is

BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWINGS

Fig. 1 illustrates bioactive compounds from *D. carota* seeds and 2,4,5-trimethoxybenzoicacid (5).

Fig. 2 is a bar graph showing *in vitro* COX-I and COX-II inhibitory activities of compounds 1-4 and 2,4,5-trimethoxybenzoic acid (5) at 100 μg mL^{-1} and Ibuprofen, Naproxen, Asprin, Celebrex and Vioxx at 2.06, 2.52, 180, 1.67 and 1.67 μg mL^{-1}, respectively. Vertical bars represent the standard deviation of each data point (n=2).

DETAILED DESCRIPTION OF THE INVENTION

The present invention is a method of treating cyclooxygenase enzyme, preferably COX-II enzyme or COX-I mediated inflammation or inflammation pain. In a preferred form of the present invention, the method treats COX II enzyme mediated inflammation or inflammation pain. In one embodiment, the invention comprises the step of providing a patient with a sufficient amount of carrot seed or carrot seed extract, wherein the patient's inflammation or pain is reduced. In a particularly advantageous form of the invention, the patient is afflicted with arthritis or gout related pain.

In the present invention, one would first identify a patient with inflammation and inflammation-related pain. In addition to arthritis or gout, other examples of inflammation-related pain would be exercise, injury, surgery, headaches, and menstrual pain.

In one embodiment, the patient would be supplied with carrot seeds, preferably in a dose of 10-400 grams per day, preferably 20-200 grams per day. Applicants envision that the carrot seeds do not necessarily need to be intact for

A "carrot seed extract" of the present invention is an extract taken from carrot seed that will preferably contain concentrated versions of the anti-inflammatory compounds 1-3 described below. The compounds may not be at the same ratio as are normally found in carrot seed and may not all be present. Preferably, 1 kg carrot seed would yield 125 grams of extract free from hexane. If triglycerides are removed, the total weight of active ingredients may be between 3-5 grams.

In another form of the invention, one would treat the patient with COX-II enzyme-mediated inflammatory pain with a mixture of compounds selected from the group consisting of 2,4,5-trimethoxybenzaldehyde, oleic acid, and trans-asarone. In a particularly adventageous form of the invention, one would treat the patient with 2,4,5-trimethoxybenzaldehyde.

In another form of the present invention, one would combine the carrot seeds, powdered carrot seeds or carrot seed extract with a second pain relieving compound, such as the compositions described below.

EXAMPLES

1. In general

The examples below disclose that cyclooxygenase enzymes inhibitory assay directed investigation of *Daucus carota* seed extracts resulted in the isolation and characterization of compounds, 2,4,5-trimethoxybenzaldehyde (1), oleic acid (2), *trans*-asarone (3) and geraniol (4). Compounds 1–4 showed 3.32, 45.32, 46.15, and 3.15% of prostaglandin H endoperoxide synthase-I (COX-I) inhibitory activity and 52.69, 68.41, 64.39 and 0% prostaglandin H endoperoxide synthase-II (COX-II) inhibitory activity, respectively at 100 μg mL^{-1}. Compound 1 showed selectivity

well as Ibuprofen and Naproxen were purchased from Sigma-Aldrich Chemical Co., Inc. St. Louis, MO). Celebrex® capsules and Vioxx® tablets were physician's professional samples provided by Dr. Subhash Gupta, Sparrow Pain center, MI.

Extraction and Isolation

The ground carrot seeds (1 kg) were sequentially extracted with hexane, EtOAc, and MeOH (1.5 L x 4, 24 h each) and yielded 121.1, 57.8 and 25.1 g of residue, respectively, after evaporating the solvents. An aliquot of the hexane extract (32 g) was stirred with MeOH and filtered to yield MeOH soluble (4.84 g) and insoluble (26 g) fractions. The bioactive MeOH soluble fraction (3.09 g) was further fractionated by MPLC on silica gel (Sanki Engineering Ltd., Model LBP-V pump operating at 1-15 psi., Chemco MPLC tayperling type glass column, 35 x 4 cm^2) using hexane with increasing amount of acetone and finally with MeOH as eluting solvents. Fractions collected were A eluted with 100% hexane and hexane:acetone (8:1, 720 mL, 220 mg), B (155 mL, 1843 mg) and C (75 mL, 145 mg) with hexane:acetone (8:1), D (75 mL, 74 mg), E (75 mL, 15 mg) and F (75 mL, 17 mg) with hexane:acetone (4:1), G (195 mL, 165 mg) with hexane: acetone (4:1 and 1:1) and H (165 mL, 300 mg) with 100% acetone and MeOH.

Compound **1** (7.8 mg) was yielded from the purification of fraction H (40 mg) by preparative TLC using hexane:acetone (4:1 x 3) as the mobile phase. Similarly, compound **2** was purified from fraction G by preparative TLC (hexane: EtOAc, 3:1). Fraction B was further subjected to MPLC on silica gel using hexane with increasing amount of acetone and yielded six fractions (I-VI). The fraction IV (73 mg), eluted with hexane:acetone (15:1), was further purified by preparative TLC (hexane:chloroform:toluene:MeOH, 3:2:2:0.1) and afforded pure compound **3** (5.9

ram seminal vesicles. PGHS-II or COX-II enzyme for the assay was prepared from lysates of cloned insect cell with human PGHS-II enzyme. Each assay mixture contained 600 µL of 0.1 M Tris buffer (pH 7), 1 mM phenol, 17 µg hemoglobin and 10 µL of COX-I or 20-30 µL of COX-II enzymes. Crude extracts or pure compounds were preincubated with 25-100 µg mL^{-1} in DMSO for 5-minutes with COX-I or COX-II enzymes in the assay chamber at 37°C. Cyclooxygenase inhibitory activities were initiated by the addition of 1.64 µM arachidonic acid to the tst compound-enzyme mixture at 37°C. Instantaneous inhibition of the enzyme was determined by monitoring the initial rate of O_2 uptake using an O_2 electrode. Ibuprofen, Naproxen and Aspirin were assayed at their IC_{50} values, 2.52, 2.06 and 180 µg mL^{-1} concentrations, respectively, whereas Celebrex and Vioxx were tested at 1.67 µg mL^{-1}.

3. Results

The ^{1}H-NMR of compound **1** gave a singlet at 10.3 ppm, which was not exchangeable with D_2O suggested that it contained an aldehydic proton in the molecule. Three singlets at 3.86, 3.91 and 3.96 ppm, integrated for three protons each, indicated the presence of three methoxy groups. In addition, two singlets at 6.47 and 7.31 ppm suggested the presence of a tetra substituted aromatic ring in the molecule. The ^{1}H NMR spectrum of **1** was identical to the published spectra of 2,4,5-trimethoxybenzaldehyde (Nowamaki and Kuroyanagi, 1996).

^{1}H NMR spectral data were sufficient to determine the structure of compound **2**. A multiplet at 5.35 ppm, integrated for two protons suggested the presence of a double bond in the molecule. Protons resonated between δ 2.34 and 1.61 were assigned to methylene protons of α- and β- to a carboxylic group. The $-CH_2$ protons

The commercially available and synthetic compounds, 2,4,5-trimethoxybenzaldehyde; 2,4,5-trimethoxybenzoicacid (**5**) and *trans*-asarone purchased from Sigma-Aldrich Chemical Co., Inc. were also tested for COX-I and COX-II inhibitory activities at 25-100 μg mL^{-1} concentrations. Synthetic 2,4,5-trimethoxybenzaldehyde showed slightly lower COX-II activity than natural form (**1**) isolated from carrot seeds, whereas COX-I and COX-II inhibition of compound **3** and synthetic *trans*-asarone were similar to natural products isolated from carrot seeds. The lower activity of the synthetic compound **1** was accounted to the presence of small amount of corresponding acid due to oxidation under storage. 2,4,5-trimethoxybenzoicacid didn't exhibit any inhibition of COX-I or COX-II enzymes at 100 μg mL^{-1} concentration.

4. Discussion

Compounds **2** and **3** isolated from carrot seeds showed comparable COX inhibition to some of the over the counter (OTC) anti-inflammatory drugs. Compound 1 exhibited selective inhibition of COX-II enzyme. The COX-II/COX-I ratio for compound 1 was 17.68 at a test concentration of 100 μg mL^{-1} compared to the solvent control. This value is better than the COX-II/COX-I ratios for Ibuprofen, Naproxen, Aspirin and Celebrex at their respective test concentrations. Compound **4** did not inhibit COX-I or -II enzymes at 100 μg mL^{-1} concentration. Among the authentic samples tested, only 2,4,5-trimethoxybenzaldehyde and *trans*-asarone exhibited COX enzyme inhibitory activities. The high COX-II/COX-I ratio of compound 1, moderate COX enzymes inhibitory activity of 3 and the lack of activity of 2,4,5-trimethoxybenzoicacid (**5**) suggested that the methoxy groups are not a major contributing factor for activity. Greca, et al. (1992) studied the structure-

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CLAIMS

We Claim:

1. A method of treating cyclooxygenase enzyme-mediated inflammation, comprising the step of treating an inflammation patient with a sufficient amount of carrot seed or carrot seed extract, wherein inflammation is reduced.

2. A method of treating COX I or COX-II enzyme mediated inflammation, comprising the step of treating an inflammation patient with a sufficient amount of carrot seed or carrot seed extract, wherein inflammation is reduced.

3. The method of Claim 2, wherein the inflammation is mediated by COX-II.

4. The method of claim 1, wherein the patient suffers from pain related to a disease selected from arthritis or gout.

5. The method of claim 1, wherein the patient is treated with carrot seeds at a dosage of 10-50 mg active ingredients.

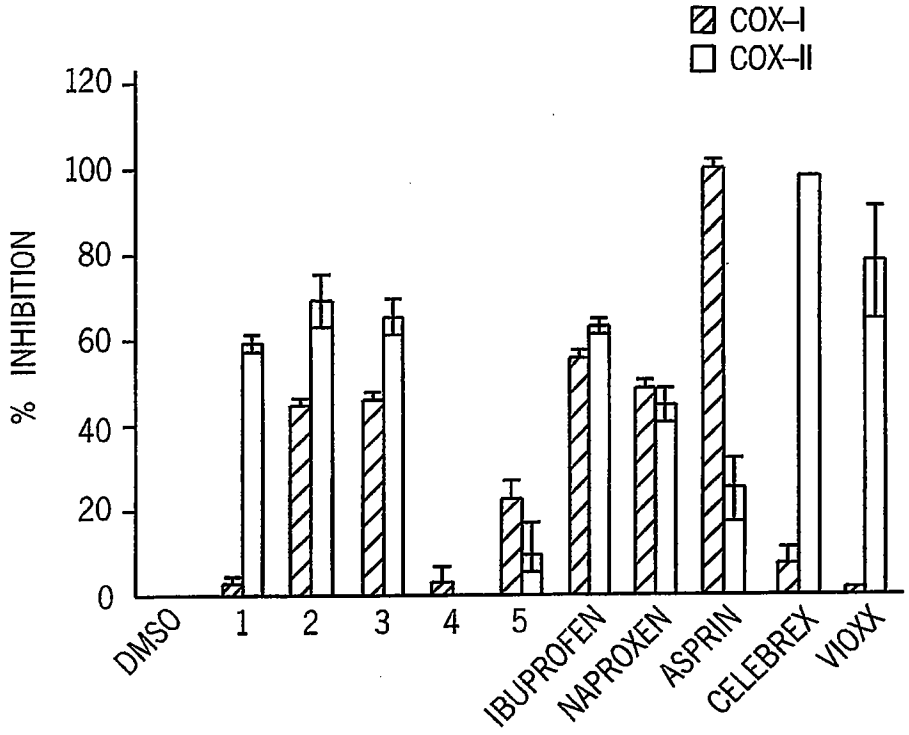
6. The method of claim 1 wherein the patient is treated with intact carrot seeds.

7. The method of claim 1 wherein the patient is treated with carrot seeds that are not intact.

16. A composition useful for the treatment of inflammation or pain wherein the composition comprises carrot seed extract.

17. The composition of claim 16 wherein the active ingredients are at 10-50 mg per dose.

18. The composition of claim 16 additionally comprising a second pain-relieving compound.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ASPRIN
CELEBREX
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FIG. 2

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Declarations under Rule 4.17:
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— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii)) for all designations*

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